EPA Reviewer: Lisa Austin, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/30/08

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HED Executive Summary Cover for the attached OECD Formatted DATA EVALUATION RECORD

STUDY TYPE: Short-term oral (90-day rat) feeding study; OPPTS 870.3100 [§82-1a] (rodent); OECD 408.

PC CODE: 118203 **DP BARCODE:** D349929

TEST MATERIAL (PURITY): BAS 800 H (93.9%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449, saflufenacil

CITATION: Kaspers, U., Strauss, V., Kaufmann, W. & van Ravenzwaay, B. (2007). BAS 800 H – Repeated Dose 90-day Oral Toxicity study in Wistar rats; Administration in the diet. Experimental Toxicology and Ecology, BASF Aktiengesellschaft 67056 Ludwigshafen, FGR. Report Number(s) 50S0414/01156. May 2, 2007. MRID 47128109. Unpublished.

SPONSOR: BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, FRG.

EXECUTIVE SUMMARY:

In a 90-day toxicity study (MRID 47128109), BAS 800 H (93.9%, batch lot#) was administered in the diet daily to Wistar rats, 10/sex/group, at nominal concentrations of 0, 50, 150, 450 (♂), 1350, or 4050 (♀) ppm (♂ = 0, 3.5, 10.5, 32.3, 94.7; ♀ = 0, 4.3, 12.6, 110.5, 344.7, respectively).

At 4050 ppm (♀ only), two females died. Clinical signs of toxicity observed included reduced general condition, severe skin paleness, anogenital urine smear, and piloerection. Food consumption was significantly reduced and there was significant reduction in body weight and body-weight gain. Due to the severe toxicological effects, this dose level was terminated on day 53. There were no treatment-related findings at 50 or 150 ppm. No clinical signs of toxicity were observed at <450 ppm. At 450 ppm (♂ only), treatment-related findings were increased spleen weight (13%) correlated with extramedullary hematopoiesis (4 vs 1/10 in controls). At 1350 ppm, males showed isolated incidences of skin paleness and piloerection, reduced motor activity, decreased body weight and body weight gain (7-14%), lowered food consumption (8-11%), increased absolute and relative spleen weight (116-137%) correlated with marked extramedullary hematopoiesis (4 vs 1/10 in controls). Females exhibited anogenital urine smears and increased spleen weight (16%) correlated with marked extramedullary hematopoiesis (3-5 vs 1/10 in controls) and increased iron storage. Affected hematological and clinical chemistry parameters were decreased hemoglobin (16-26%, ♂♀), hematocrit (14-17%, ♂♀), mean corpuscular volume (MCV) (13-15%, ♂♀), mean corpuscular hemoglobin (MCH) (16-25%, ♂♀), mean corpuscular hemoglobin concentration (MCHC) (3-12%, ♂♀), total protein and globulins (5-6%, ♂),

increased normoblasts and reticulocytes (♂,205%%; ♀, 38%), total bilirubin (59%), urinary transitional epithelial cells (9-10 vs 3/10 in controls >2, ♂), and urinary casts (10 vs 2/10 in controls >1, ♂).

The LOAELs established in males and females were 450 (32.3 mg/kg bw/d) and 1350 ppm (110.5 mg/kg bw/d), respectively, based on multiple hematological effects and histopathology of the spleen. A NOAEL of 150 ppm (♂ = 10.5, ♀ = 12.6 mg/kg bw/d) was established.

This 90-day oral toxicity study in the rat is an acceptable guideline study and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and Data Confidentiality statements were provided.

This Executive Summary was prepared for the United States Environmental Protection Agency, Office of Pesticide Program, Health Effects Division Use.

Much of the text was generated by the submitter(s) in OECD format. However, this document has undergone critical scientific analysis in comparison to the study report and modified as needed.

Reviewer #: Steve Wong, Ph.D., Date: April 16, 2008

APPLICANT: BASF Corporation

STUDY TYPE: Short-term (90-day rat) dietary study; OPPTS 870.3100 (rat); OECD 408.

TEST MATERIAL (PURITY): BAS 800 H (93.9%)

SYNONYMS: AC 433379; BASF Reg. No. 4054449

CITATION: Kaspers, U., Strauss, V., Kaufmann, W. & van Ravenzwaay, B. (2007). BAS 800 H – Repeated Dose 90-day Oral Toxicity study in Wistar rats; Administration in the diet. Experimental Toxicology and Ecology, BASF Aktiengesellschaft 67056 Ludwigshafen, FGR. Report Number(s) 50S0414/01156. BASF Doc ID 2005/1012914. May 2, 2007. Unpublished. [PMRA# 1547000]

SPONSOR: BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, FRG

EXECUTIVE SUMMARY:

In a 90-day dietary toxicity study, BAS 800 H (93.9%) was administered daily in the diet to Wistar rats, 10/sex/group, at 0, 50, 150, 450 (♂), 1350, or 4050 (♀) ppm (♂ =0, 3.5, 10.5, 32.3, 94.7; ♀ = 0, 4.3, 12.6, 110.5, 344.7, respectively). At 4050 ppm (♀ only), two females died. Clinical signs of toxicity observed included reduced general condition, severe skin paleness, anogenital urine smear, and piloerection. Food consumption was significantly reduced and there was significant reduction in body weight and body-weight gain. Due to the severe toxicological effects, this dose level was terminated on day 53. There were no treatment-related findings at 50 or 150 ppm. No clinical signs of toxicity were observed at ≤450 ppm. At 450 ppm (♂ only), treatment-related findings were increased spleen weight correlated with extramedullary hematopoiesis. At 1350 ppm, males showed isolated incidences of skin paleness and piloerection, reduced motor activity, decreased body weight and body weight gain, lowered food consumption, increased spleen weight correlated with marked extramedullary hematopoiesis. Females exhibited anogenital urine smears and increased spleen weight correlated with marked extramedullary hematopoiesis. Systemic effects were porphyria involving erythrocytes in box sexes. Affected hematological and clinical chemistry parameters were decreased hemoglobin (♂♀), hematocrit (♂♀), mean corpuscular volume (MCV) (♂♀), mean corpuscular hemoglobin (MCH) (♂♀), mean corpuscular hemoglobin concentration (MCHC) (♂♀), total protein and globulins (♂), increased normoblasts and reticulocytes (♂♀), increased chloride, total bilirubin, urinary transitional epithelial cells, and urinary casts (♂). Based on multiple hematology endpoints typical of anemia and histopathology of the spleen the LOAELs established in males and females were 450 (32.3 mg/kg bw/d) and 1350 ppm (110.5 mg/kg bw/d), respectively. A NOAEL of 150 ppm (♂ = 10.5, ♀ = 12.6 mg/kg bw/d) was established.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

51

I. MATERIALS AND METHODS

A. MATERIALS:

1.

Test material:	BAS 800 H
Description:	Solid / bright-beige; stored at room temperature
Lot/Batch #:	COD - 000298
Purity:	93.9% a.i.
Compound stability:	The stability under the storage conditions present in this study was guaranteed by the Certificate of Analysis. The homogeneity of the test material was confirmed by analysis.
CAS #:	372137-35-4

2. **Vehicle and/or positive control:** The test substance was administered in the diet.

3. **Test animals:**

Species:	Rats	
Strain:	CrlGlxBrlHan:WI	
Age/weight at study initiation:	Age: 32±1 day ; Mean weight: ♂ = 152.1; ♀ = 120.5 g	
Source:	Charles River, Germany, Sandhofer Weg 7, 97633 Sulzfeld	
Housing:	singly in DK III stainless steel wire mesh cages (floor area about 800 cm^2)	
Diet:	Kliba maintenance diet mouse/rat "GLP", meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	20-24°C 30-70% no information 12h dark / 12h light
Acclimation period:	At least five days prior to administration (based on animal arrival and initiation of dosing information)	

B. STUDY DESIGN:

1. **In life dates:** Start: September 10, 2004 End: December 14, 2004

2. **Animal assignment:** Animals were assigned to test groups via a randomization protocol provided by a computer. The test groups are noted in Table 1.

Table 1: Study design

	♂					♀				
ppm	0	50	150	450	1350	0	50	150	1350	4050
mg/kg bw	0	3.5	10.5	32.3	94.7	0	4.3	12.6	110.5	344.7
N	10	10	10	10	10	10	10	10	10	10

3. **Diet preparation and analysis:**

For each concentration, the test substance was weighed out and mixed with a small amount of food. Appropriate amounts of food, depending on dose group, were added to this premix in order to obtain the

desired concentrations. The test substance preparations were usually mixed every four weeks. The stability of BAS 800 H in the diet was proven with a comparable batch of test substance for a period of up to 49 days at room temperature. Homogeneity analyses of the BAS 800 H preparations were performed in samples of all concentrations at the start and end of the administration period. These samples also served as the concentration control analyses.

Results

Homogeneity and concentration analysis: The mean concentrations ranged from 91.3 to 103.9% of the nominal concentrations, with low standard deviations (never greater than 5.5%).

Stability analysis: In feed, BAS 800 H was stable for a period of 49 days (0 D: 100.0% of nominal; 9 D: 102.7% of nominal; 34 D: 95.3% of nominal; 49 D: 97.9% of nominal).

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the rats was acceptable.

Statistics:

Parameter	Statistical Test*	References
Food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group using the Dunnett-test (2-sided) for the hypothesis of equal means	Dunnett, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 - 1121 Dunnett, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 - 491
Feces, rearing, grip strength length forelimbs, grip strength length hind limbs, foot-splay test, motor activity Clinical pathology parameters, except reticulocytes and differential blood count	Non-parametric one-way analysis using Kruskal-Wallis test (2-sided).If p≤0.05, a pair-wise comparison of each dose group with the control group was performed using Wilcoxon-test (2-sided) for the equal medians	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York
Weight Parameters	Non-parametric one-way analysis using Kruskal-Wallis-test (2-sided). If p≤0.05, a pairwise comparison of each dose group with the control group was performed using the Wilcoxon-test (2-sided) for the equal medians	Miller, R. G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3. Nijenhuis, A. and Wilf, H.S. (1978): Combinatorial Algorithms, Academic Press, New York, 32-33. Hettmannsperger, T. P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132- 140.
Urinalysis, except volume, color, turbidity and specific gravity	Pair-wise comparison of each dose group with the control group using Fisher's exact test for the hypothesis of equal proportions	Siegel S. (1956): Non-parametric statistics for behavioral sciences. McGraw-Hill New York
* Significantly different (p <0.05) from the control. ** Significantly different (p <0.01) from the control.		

C. METHODS:

1. Observations:

The rats were examined for signs of toxicity or mortality twice a day on weekdays and once a day on Saturdays, Sundays and public holidays.

Detailed Clinical Observations (DCO):
Detailed clinical observations were conducted for all rats prior to initiation of dosing and thereafter at weekly intervals. Parameters examined were as follows:

abnormal behavior during handling		activity / arousal level		palpebral closure
feces (appearance/consistency)		tremors	urine	exophthalmus
skin	fur	convulsions	lacrimation	abnormal movements
posture	pupil size	salivation	respiration	impairment of gait

Functional Observational battery (FOB):
A FOB was conducted for all rats at the end of the administration period.

Home cage observations:

posture	convulsions	tremor	abnormal movements	impairment of gait	other findings

Open field observations:

fur	eyes/pupil size	palpebral closure	abnormal movements	activity/arousal level
salivation	posture	nose discharge	feces within two minutes	urine within two minutes
respiration	skin	impairment of gait	number of rearings within two minutes	
lacrimation	tremors	convulsions	behavior when removed from cage	

Sensorimotor Tests/Reflexes:

approach response	pinna reflex	landing foot-splay test	audition (startle response)
touch response	vocalization	grip strength – forelimbs	grip strength – hindlimbs
vision	pain perception (tail pinch)	behavior during handling	
papillary reflex	coordination (righting response)		other findings

Motor activity assessment:
Motor activity was measured on the same day as the FOB. Motor activity was assessed for 60 minutes in the dark using a Multi-Varimex-System (Columbus Instruments Int. Corp., USA).

2. Body weight:

Body weight was determined before the start of the administration period in order to randomize the animals. The weights were then determined on day 0 and weekly thereafter.

3. Food and consumption and compound intake:

Food consumption for each animal was determined weekly, and mean daily diet consumption was calculated as g food/kg bw/d. Food efficiency (body weight gain in g/food consumption in g per unit time X 100) and compound intake (mg/kg bw/d) values were calculated as time-weighted averages from the food consumption and body weight gain data. Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

4. Ophthalmoscopic examination:

Prior to the administration period, the eyes of all rats were examined with an ophthalmoscope. Eyes of the control and high dose (1350 ppm) rats were examined with an ophthalmoscope at the end of the study (Day 91)

5. Hematology & clinical chemistry:

At the end of the dosing period (days 94/95), blood was removed from fasted animals from the retro-orbital venous plexus or after decapitation. The CHECKED (X) parameters were examined.

a. **Hematology:**

x	hematocrit (HCT)*	x	hemoglobin (HGB)*	x	leukocyte differential count*
x	leukocyte count (WBC)*			x	mean corpuscular HGB (MCH)*
x	erythrocyte count (RBC)*			x	mean corpuscular HGB conc.(MCHC)*
x	blood clotting measurements*			x	mean corpuscular volume (MCV)*
x	platelet count			x	reticulocyte count
x	clotting parameters - prothrombin time (thromboplastin time, clotting time)				
* Recommended for subchronic rodent studies based on Guideline 870.3100					

b. Clinical chemistry:

ELECTROLYTES						OTHER	
x	calcium*	x	chloride*	x	potassium*	x	albumin*
x	phosphorus*	x	magnesium	x	sodium*		blood creatinine*
ENZYMES						x	blood urea nitrogen*
x	alkaline phosphatase (AP)		cholinesterase (ChE)			x	total cholesterol
x	creatine phosphokinase		glutamate dehydrogenase			x	globulins
x	serum alanine amino-transferase (ALT/ SGPT)*					x	glucose*
x	serum aspartate amino-transferase (AST/ SGOT)*					x	total bilirubin*
x	gamma glutamyl transferase (GGT)*					x	total serum protein (TP)*
	lactic acid dehydrogenase (LDH)					x	triglycerides
	ornithine decarboxylase*						serum protein electrophores
* Recommended for subchronic rodent studies based on Guideline 870.3100							

6. Urinalysis:

For urinalysis, assessed at study termination, individual rats were transferred to metabolism cages (withdrawal of food and water) and urine was collected overnight. The urine samples were evaluated in a randomized sequence. The following parameters were analyzed:

x	pH	x	protein	x	urobilinogen	x	blood
x	glucose	x	ketones	x	bilirubin	x	specific gravity
x	sediment	x	volume	x	color, turbidity		

7. Sacrifice and pathology:

All rats that died and those sacrificed on schedule by decapitation under CO_2 anesthesia. were subjected to gross pathological examination and the CHECKED (x) tissues were collected for histological examination. For the low- and mid-dose animals, only the liver, bone marrow, spleen, and gross lesions were processed for histopathological examination. The (xx) organs were weighed.

Digestive system		Cardiovascular/Hematologic				Neurological system			
	tongue	x	aorta*	xx	spleen*+	xx	brain*+	x	pituitary*
x	salivary glands*	xx	heart*+	xx	thymus*+	x	peripheral nerve*		
x	esophagus*	x	bone marrow*			x	spinal cord (3 levels)*		
x	stomach*	x	lymph nodes*			x	eyes (optic nerve)*		
x	duodenum*	Urogenital system				Glandular organs			
x	jejunum*	xx	kidneys*+	xx	testes*+	x	adrenal gland*+		
x	ileum*	xx	ovaries*+	xx	uterus*+	x	lacrimal gland[t]		
x	cecum*	x	urinary bladder*			x	mammary gland*		
x	colon*	xx	epididymides*+			x	parathyroid*		
x	rectum*	x	prostate*			xx	thyroid*		
xx	liver*+	x	seminal vesicles*			Others			
x	gall bladder*	x	oviducts, uterus and vagina			x	bone	x	skin
x	pancreas*	Respiratory system				x	skeletal muscle		
		x	trachea*	x	nose*	x	all gross lesions and masses*		
		x	lungs*	x	larynx*	x	target organs*		
			pharynx*			xx	anesthetised animals		

* Recommended for subchronic rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies; T = required only when toxicity or target organ

II. RESULTS

A. Observations:

1. Clinical signs of toxicity:

At 4050 ppm, all females showed signs of systemic toxicity such as skin paleness (severe, from day 35), piloerection (from day 49), reduced general condition (slight to moderate, from day 49) and urine smeared anogenital region (slight, moderate or severe, from day 7 onwards). After two females at 4050 ppm were found dead on study day 53, the remaining females in this group were sacrificed ahead of schedule.

At 1350 ppm, urine smeared anogenital region was observed in two females. Two males showed skin paleness (slight) on day 91, one showed piloerection on day 91.

2. Mortality:

Two females at 4050 ppm were found dead on day 53. Because of the obviously exceeded maximum tolerated dose (MTD) and due to a deteriorating general condition of the surviving eight females, the remaining rats at this dose group were sacrificed on day 53.

57

B. Body weight and weight gain:

At 4050 ppm body weight and body weight changes were statistically significantly decreased in females. At 1350 ppm body weight and body weight of the males were decreased during the whole study period, the decrease was statistically significant during days 21 to 49 periods.

Table 2. Average body weights (g±SD) and body weight gains during 91 days of treatment

	♂ (N = 10/group)					♀ (N = 10/group)				
ppm	0	50	150	450	1350	0	50	150	1350	4050
mg/kg bw/d	0	3.5	10.5	32.3	94.7	0	4.3	12.6	110.5	344.7
Day 0	153.4 ±7.1	152.5 ±6.2	152.2 ±7.1	152.2 ±6.5	150.3±7.7	120.7 ±5.0	120.7 ±4.8	119.4 ±5.1	121.4 ±6.1	120.1±5.6
Day 14	234.1 ±11.5	224.3 ±26.2	232.4 ±11.6	231.5 ±11.9	220.1±14.0 (-6.0%)	155.2 ±6.9	147.9 ±9.8	150.6 ±12.0	153.2 ±9.5	145.2±9.6 (-6.5%)
Day 21	264.4 ±14.9	258.7 ±15.5	263.5 ±15.1	259.8 ±15.3	**245.2±16.5*** **(-7.3%)**	167.3 ±13.0	162.8 ±11.3	161.6 ±16.0	168.6 ±10.4	157.3±7.5 (-6.0%)
Day 28	288.8 ±16.3	281.0 ±19.6	284.7 ±17.3	280.4 ±17.6	**265.2±20.0*** **(-8.2%)**	177.9 ±11.7	175.6 ±11.2	171.8 ±15.4	179.2 ±10.7	**164.6±8.5*** **(-7.5%)**
Day 35	309.9 ±19.0	299.7 ±23.7	304.6 ±19.9	296.8 ±15.8	**282.2±20.7*** **(-8.9%)**	184.6 ±12.0	180.7 ±13.5	179.2 ±15.4	187.3 ±11.2	**169.9±7.2*** **(-7.9%)**
Day 49	330.6 ±20.9	322.3 ±27.4	325.5 ±23.3	320.9 ±23.4	**303.1±23.2*** **(-8.3%)**	198.3 ±13.1	193.8 ±1715	191.2 ±20.1	198.4 ±12.0	**161.4±17.0**** **(-18.6%)**
Day 56	344.5 ±22.6	338.3 ±29.1	341.8 ±26.1	336.9 ±25.1	318.7±26.2 (-7.5%)	204.7 ±13.3	203.6 ±14.9	198.5 ±21.2	205.8 ±11.0	-
Day 70	363.8 ±22.4	351.7 ±28.6	360.1 ±30.6	353.2 ±28.7	340.4±28.4 (-7.9%)	212.8 ±13.7	205.1 ±13.6	207.2 ±19.4	208.2 ±14.9	-
Day 91	376.8 ±19.9	364.6 ±34.5	375.9 ±31.6	363.5 ±32.5	342.3±32.2 (-9.2%)	215.2 ±15.2	210.8 ±12.5	210.1 ±20.0	213.1 ±15.8	-
Change Day 0-91	223.4 ±14.9	212.1 ±33.5	223.7 ±28.7	211.4 ±31.2	**191.9±25.8*** **(-14.1%)**	94.5 ±11.2	90.1 ±9.5	90.7 ±16.1	91.8 ±12.7	-

Data taken from Table 1A. pages 73-76 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

C. Food consumption and compound intake:

1. Food consumption:

Males at 1350 ppm had lower food consumption during most of the study period, statistically significantly from day 7 to 49. Food consumption was also statistically significantly reduced in females at 4050 ppm.

Table 3. Mean food consumption (g/rat/d ± SD) during 91 days of treatment

	♂ (N = 10/group)					♀ (N = 10/group)				
ppm	0	50	150	450	1350	0	50	150	1350	4050
mg/kg bw/d	0	3.5	10.5	32.3	94.7	0	4.3	12.6	110.5	344.7
Day 7	20.3±0.9	20.4±0.7	20.3±1.5	20.4±1.1	**18.6±1.2****	15.0±1.0	15.1±1.0	14.6±0.9	15.2±1.2	14.1±1.2
Day 14	21.7±0.9	20.5±3.8	21.6±1.6	21.6±1.4	19.9±1.0	15.4±1.0	14.8±0.9	14.8±1.2	15.0±1.3	**13.7±1.0****
Day 21	22.6±1.3	22.5±1.5	22.6±1.5	22.4±1.4	**20.2±1.2****	16.5±2.3	15.9±1.2	15.1±1.5	15.6±1.0	**14.3±0.8****
Day 28	22.5±1.2	21.9±1.4	21.8±1.3	21.7±1.8	**20.2±1.2****	15.1±1.6	16.0±1.0	15.4±1.2	15.9±0.9	14.4±0.8
Day 35	22.8±1.2	21.5±1.7	22.1±1.3	22.0±1.2	**20.3±1.2****	16.1±1.3	15.7±2.0	15.5±1.4	15.8±0.8	**14.0±0.6****
Day 49	21.9±1.1	21.5±1.8	21.4±1.4	21.9±1.5	**20.1±1.4***	16.1±1.2	16.7±2.3	15.7±1.7	16.0±0.8	**10.5±3.2****
Day 56	21.2±1.3	21.3±1.5	21.4±2.0	21.8±1.8	20.4±1.5	16.2±1.6	16.3±0.8	15.7±1.6	15.4±0.7	-
Day 70	21.3±0.8	19.9±1.3	20.8±1.8	20.9±1.7	20.1±1.6	15.7±1.1	15.6±0.9	15.5±1.3	15.0±1.0	-
Day 91	21.3±1.3	21.0±1.4	21.4±1.8	21.9±1.9	20.4±2.3	16.0±1.4	16.3±1.2	16.0±1.3	15.5±1.1	-

Data taken from Table IA. pages 69-72 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

2. Compound consumption: See Table 1 for the mean daily test substance intake in mg/kg bw/d.

57

3. Food efficiency:

Food efficiency of females at 4050 ppm was statistically significantly decreased on day 49. There were a few statistically significantly decreased values in other dose groups at isolated intervals. Due to the isolated occurrences and the lack of a dose-response relationship, these findings were assessed as not compound-related.

D. Ophthalmoscopic examination:

All findings were judged to be incidental in nature, due to the occurrence in single animals, and/or lack of a dose-response relationship.

E. Blood analyses:

1. Hematology:

Statistically significantly decreased values for hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) at 1350. Hemoglobin, Hct, MCV and MCH values were also decreased in males at 450 ppm. The slightly higher platelet counts and shortened prothrombin times in females at 1350 ppm, although marginally statistically significant, were probably incidental findings. Increased numbers of white blood cells were observed in male and female rats at 1350 ppm. In the differential blood count acquired with the automated hematology analyzer the increase in leukocytes was associated with an increase in lymphocytes. The increases in white blood cells were not caused by elevated lymphocytes but by an increased number of normoblasts, which were incorrectly identified as leukocytes based on size measurements alone.

Table 4. Selected hematological data (mean±SD; assessed on day 94/95)

	♂ (N = 10/group)					♀ (N = 10/group)			
ppm	0	50	150	450	1350	0	50	150	1350
mg/kg bw/d	0	3.5	10.5	32.3	94.7	0	4.3	12.6	110.5
Hb	9.5 ±0.3	9.4 ±0.3	9.2 ±0.3	**8.6**** ±0.4	**7.0**** ±0.7	8.9 ±0.3	9.0 ±0.2	8.8 ±0.3	**7.5**** ±0.3
Hct, %	42.1 ±1.8	42.3 ±1.1	41.6 ±1.5	**38.8**** ±0.9	**35.0**** ±2.4	40.4 ±0.8	40.1 ±1.2	40.2 ±2.0	**34.8**** ±1.2
MCV, fL	51.0 ±1.8	51.3 ±1.0	50.8 ±1.6	**47.0**** ±2.2	**43.2**** ±1.6	53.5 ±1.1	53.8 ±1.7	53.2 ±1.7	**46.3*** ±2.5
MCH, fmol	1.15 ±0.05	1.13 ±0.04	1.13 ±0.05	**1.04**** ±0.07	**0.86**** ±0.04	1.18 ±0.05	1.20 ±0.04	1.16 ±0.05	**0.99**** ±0.08
MCHC, mmol/L	22.5 ±0.48	22.1 ±0.52	22.2 ±0.36	22.1 ±0.57	**19.9**** ±0.57	22.1 ±0.62	22.3 ±0.41	21.9 ±0.57	**22.4*** ±0.61
WBC, 10^9/L	5.55 ±1.00	4.86 ±1.06	5.57 ±1.29	5.42 ±1.03	**9.94**** ±2.61	3.84 ±0.52	3.70 ±0.41	3.47 ±1.22	**4.95**** ±1.28
Reticulocytes, %	1.9±0.36	2.0±0.3	2.1±0.3	2.0±0.3	**5.8±2.7**	2.4±0.4	2.3±0.4	2.4±0.3	**3.3±1.1**

Data taken from Table IB. pages 111-124 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

2. Clinical chemistry: Table 5

There were no treatment-related effects in serum enzyme activities.

In males, blood chemistry examinations revealed statistically significantly increased chloride and total bilirubin concentrations in males at 1350 ppm and reduced total protein and globulins levels in males at

58

450 ppm and 1350 ppm. These findings were assessed to be substance related. Statistically significantly decreased total protein concentration was also found in males at 150 ppm, however, this value was within the historical control range and was not accompanied by any other hematological or pathological change. Therefore, this finding was not considered treatment related and/or biologically relevant.

In females, there were no treatment-related changes in the blood chemistry parameters.

Table 5. Selected chemistry data in males (mean±SD; assessed on day 94)

	♂ (N = 10/group)				
ppm	0	50	150	450	1350
mg/kg bw/d	0	3.5	10.5	32.3	94.7
Cl, mmol/L	105.4±1.4	106.3±1.5	106.2±1.0	106.1±1.3	**108.2±1.2****
Bilirubin, µmol/L	2.37±0.25	2.32±0.41	2.27±0.29	2.49±0.36	**3.78±0.84****
Protein, g/L	67.2±1.56	66.3±1.45	65.7±1.65*	**63.9±1.98****	**63.0±2.03****
Globulin, g/L	30.0±1.30	29.2±1.48	29.1±0.86	**27.7±1.90***	**26.0±1.87****
Data taken from Table IB, pages 111-124 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related					

F. Urinalysis: Table 6

Discolored (maize-yellow to orange) urine was observed in some males at 50, 450, and 1350 ppm and some females at 1350 ppm. Significantly increased urobilinogen levels were evident in males at 150, 450, and 1350 ppm and in females at 1350 ppm. Urinary bilirubin concentrations were also elevated in males at 450 and 1350 ppm and in females at 1350 ppm. An increased number of transitional epithelial cells was found in males at 450 and 1350 ppm and granulated casts were detected in males at 1350 ppm.

Table 6. Selected urinalysis data (# rats affected; assessed on day 83)

		♂ (N = 10/group)					♀ (N = 10/group)			
ppm		0	50	150	450	1350	0	50	150	1350
mg/kg bw/d		0	3.5	10.5	32.3	94.7	0	4.3	12.6	110.5
Colour	Normal, yellow clear	10	9	6	2		10	10	9	4
	Yellow-orange clear		1		2	7			1	
	Maize yellow colour			4	6	3				6
Urobilinogen	≤17 µmol/L	8	3	2	0	0	8	5	6	1
	≥68 µmol/L	2	7	8*	**10****	**10****	2	5	4	**9****
Bilirubin	≤9 µmol/L	10	10	9	3	2	10	10	10	7
	≥25 µmol/L	0	0	1	**7****	**8****	0	0	0	3
Transitional cells	≤1	7	8	9	1	0	10	10	10	10
	≥2	3	2	1	**9****	**10****	0	0	0	0
Casts	0	8	8	8	7	0	10	10	10	10
	≥1	2	2	2	3	**10****	0	0	0	0
Data taken from Table IB, pages 125-128 and Table IIB, pages 250-259 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related										

G. Sacrifice and pathology:

1. Organ weight: Table 7

In males at 1350 ppm, treatment-related effects on organ weights were noted for the spleen and heart, organs associated with treatment-related anemia. Other statistically significant organ weight values were regarded to be secondary to the lower terminal body weight.

For females at 1350 ppm, a trend toward an increase in spleen weight was regarded as a treatment-related effect.

Table 7. Selected organ weight data (assessed on day 94/95)

		♂ (N = 10/group)					♀ (N = 10/group)			
ppm		0	50	150	450	1350	0	50	150	1350
mg/kg bw/d		0	3.5	10.5	32.3	94.7	0	4.3	12.6	110.5
Terminal BW, g		349.4 ±18.3	339.9 ±32.8	347.9 ±31.3	335.3 ±29.9	314.1 ±26.9	200.1 ±13.4	196.2 ±14.6	195.6 ±19.5	199.6 ±13.4
Heart	g	1.092 ±0.082	1.074 ±0.117	1.091 ±0.102	1.134 ±0.104	**1.255** ±0.123**	No treatment-related differences			
	%BW	0.313 ±0.023	0.316 ±0.021	0.314 ±0.023	0.339* ±0.018	**0.400** ±0.024**				
Spleen	g	0.564 ±0.044	0.563 ±0.039	0.586 ±0.075	0.638 ±0.090	**1.220** ±0.576**	0.374 ±0.050	0.396 ±0.030	0.399 ±0.053	**0.435 ±0.080**
	%BW	0.162 ±0.011	0.167 ±0.018	0.168 ±0.016	0.190* ±0.019	**0.385** ±0.170**	0.188 ±0.030	0.203 ±0.019	0.205 ±0.029	**0.218 ±0.039**
Liver	g	8.450 ±0.548	8.120 ±0.879	8.212 ±0.929	7.930 ±0.712	7.830 ±0.898	4.871 ±0.308	4.899 ±0.212	4.729 ±0.392	5.205 ±0.405
	%BW	2.419 ±0.107	2.387 ±0.063	2.357 ±0.095	2.366 ±0.072	2.488 ±0.120	2.437 ±0.123	2.504 ±0.122	2.422 ±0.084	2.611* ±0.180
Thyroid	mg	19.5 ±1.84	20.7 ±2.63	20.6 ±4.58	19.2 ±2.39	**16.4** ±2.07**	No treatment-related differences			
	%BW	0.006 ±0.001	0.006 ±0.001	0.006 ±0.001	0.006 ±0.0	0.005 ±0.001				
Testes	g	3.383 ±0.192	3.472 ±0.285	3.468 ±0.250	3.531 ±0.313	3.576 ±0.212				
	%BW	0.969 ±0.046	1.029 ±0.118	1.003 ±0.098	1.057* ±0.097	**1.144** ±0.090**				
Brain	g	1.995 ±0.062	2.022 ±0.070	2.036 ±0.108	2.013 ±0.060	1.988 ±0.077				
	%BW	0.572 ±0.026	0.600 ±0.067	0.588 ±0.038	0.604 ±0.043	**0.636** ±0.039**				

Data taken from pages 129-132 of Report; * ≤0.05; ** ≤0.01; bold values are considered treatment-related

2. Gross pathology:

Visibly enlarged spleens were observed in the males at 1350 ppm. This finding is regarded as treatment-related. All other gross lesions are considered spontaneous in origin and not related to treatment.

3. Microscopic pathology: Table 8

Table 8. Selected histopathological findings (# animals affected)

		♂ (N = 10/group)					♀ (N = 10/group)			
ppm		0	50	150	450	1350	0	50	150	1350
mg/kg bw/d		0	3.5	10.5	32.3	94.7	0	4.3	12.6	110.5
Liver	Iron storage, grade 1				2	1	5	3	3	6
	2					6	3	3	4	2
	3					2	2	4	2	1
	4					1			1	1
	Extramedullary hematopoiesis					5				2
Spleen	Extramedullary hematopoiesis gr 2	1			4	1	1	2	3	3
	3					6				2
	4					3				

Data taken from pages 134-137 of Report; bold values are considered treatment-related
grades 1-minimal, 2-slight, 3-moderate, 4-severe, 5-extreme

Extramedullary hematopoiesis was the main treatment-related histological finding affecting the liver of males at 1350 ppm and the spleen of males at 450 and 1350 ppm and females at 150 and 1350 ppm. When compared to the control females, the incidence and magnitude of the effects for the females at 150 ppm were comparable and the findings might not be considered toxicologically significant. Increased iron storage in the liver in males and females at 1350 ppm was also related to the test material. All other findings noted were considered as spontaneous or incidental in origin and were not related to treatment.

H. Functional Observational Battery (FOB): Table 8.

Deviations from "zero values" were obtained in several animals. However, as most findings were equally distributed among test and control groups, and were without a dose-response relationship or occurred in only single animals, these observations were considered as incidental.

Quantitative parameters: The value of the landing foot-splay test was decreased in males at 50 and 1350 ppm. The decreases were slight and were not considered to be related to the test article due to the absence of a dose-response relationship.

Home cage observations: No substance-related findings were observed.

Open field observations: Two female animals of the 1350 ppm dose group showed urine staining of the anogenital region.

Sensorimotor tests/reflexes: No substance-related effects were observed.

I. Motor activity: Table 8.

Table 8. Selected FOB and motor activity findings in males (mean±SD)

		♂ (N = 10/group)				
ppm		0	50	150	450	1350
mg/kg bw/d		0	3.5	10.5	32.3	94.7
Landing foot spray, cm		10.6±0.9	9.8±0.5*	10.2±0.6	10.1±0.5	9.7±0.6*
Motor activity, # beam interruptions	Interval 1	55.4±14.3	60.1±19.2	61.0±9.5	57.0±13.9	51.6±9.4
	Interval 2	45.4±10.5	50.3±10.5	45.7±9.6	52.7±10.6	**35.1±11.8***
	Interval 4	31.3±12.1	35.5±14.4	34.3±13.0	28.8±9.8	30.8±9.4
	Interval 6	27.3±8.7	25.8±8.5	16.7±11.0*	27.6±14.5	**13.3±11.4****
	Interval 8	21.7±18.0	17.5±17.6	8.2±8.1	16.0±14.0	7.0±13.0
	Interval 10	7.0±7.1	14.5±12.5	5.9±7.1	7.2±13.7	4.7±9.8
	Interval 12	0.5±1.3	10.1±14.1	1.5±2.8	5.2±10.5	10.2±15.6
	Total 1-12	299.5±63.1	336.6±81.3	261.9±49.5	299.8±56.6	**230.5±75.2***

Data taken from pages 103-137 of Report; * ≤0.05; ** ≤0.01; **bold** values are considered treatment-related

Statistically significantly decreased values (intervals 2, 6, and overall) were measured in males at 1350 ppm. These findings were assessed as related to the test article and caused by systemic toxicity/reduced body weight. A few isolated statistically significantly single intervals were considered incidental.

61

III. DISCUSSION

A. Authors' conclusions:

The oral administration of BAS 800 H in the diet to male and female rats over three months caused signs of general systemic toxicity as well as microcytic hypochromic anemia and porphyria. The red blood cells were recognized as a target of the test article.

The LOAEL established in males was 450 ppm (32.3 mg/kg bw/d) based on multiple clinical chemistry endpoints typical of microcytic hypochromic anemia (decreased Hb, Hct, MCV, MCH), total protein and globulins). Histopathological findings at this LOAEL were spleen weight increases with extramedullary hematopoiesis.

The LOAEL established in the females was 1350 ppm (110.5 mg/kg bw/d) based on multiple clinical hematological endpoints typical of microcytic hypochromic anemia (decreased Hb, Hct, MCV, MCH, and MCHC). Histopathological findings at this LOAEL were spleen weight increases with extramedullary hematopoiesis.

The NOAEL in both sexes was 150 ppm (♂ = 10.5, ♀ = 12.6 mg/kg bw/d).

B. Reviewer's comments:

The study was properly conducted and reported. The authors' conclusions are valid.

62